PATENT
0259-0417P

IN THE U.S. PATENT AND TRADEMARK OFFICE

APPLICANT:	Kirschner, G. et al.	CONF:	1390
SERIAL NO.:	10/663,198	GROUP:	1651
FILED:	September 15, 2003	EXAMINER:	Marx, I.

FOR: PROCEDURE FOR THE PREPARATION OF PURE PHOSPHATIDES AND THEIR USE IN THE COSMETIC, PHARMACEUTICAL AND ALIMENTARY FIELDS

DECLARATION SUBMITTED UNDER 37 C.F.R. § 1.132

Honorable Commissioner
Of Patents and Trademarks
P.O. Box 1450
Alexandria, VA 22313-1450

July 5, 2007

Sir:

I, Giampaolo Menon of the Laboratory of Analytical Development, Fidia Farmaceutici S.p.A, Italy, do hereby declare the following:

I have attached a copy of my *curriculum vitae* to this Declaration

I am a technician in the Laboratory of Analytical Development and conducted the experiments described below.

I am an inventor of the above-referenced patent application and a person ordinary skill in the phosphatide art. I am familiar with enzymatically catalyzed reactions as well as the development, usages and properties of phosphatidylcholine compounds.

I have read and understand the subject matter of the Office Action issued on January 8, 2007 in connection with the above-identified application. I have also read and understand the subject matter of the Sakai et al. patent (USPN 6,117,853).

I offer the following experimental results which show that the phosphatidyl serine sodium salt compositions presently claimed in application 10/663,198 have properties that are different than and surprisingly improved over those disclosed by the Sakai et al. patent.

In particular, I have made phosphatidylserine according to the method disclosed by Sakai et al. in Example 1 of USPN 6,117,853, which is a two part process. I conducted the first part of the process as described in Column 4, lines 20-32 of the Sakai et al. patent, and analyzed the products by thin layer chromatography (TLC). Figure 1 shows the results of this TLC experiment, which demonstrates that the first part of the Sakai process yields a mix of products comprised of approximately 40% phosphatidyl choline, 40% phosphatidyl serine, 15% phosphatidic acid and two other faint spots of uncertain identity. It is therefore my opinion that the 95% pure phosphatidyl serine presently claimed in application 10/663,198 has a different and surprisingly improved degree of purity over phosphatidyl serine produced according to the first part of the Sakai et al. process.

I have also conducted the second part of the Sakai et al. process as described in Column 4, lines 33-46 of USPN 6,117,853, and analyzed the products by TLC. Figure 2 shows the results of this TLC experiment, which demonstrates that nothing is eluted from the silica column. It is therefore clear that the second part of the Sakai et al. process, as disclosed in Column 4, lines 33-46, fails to yield phosphatidylserine at all.

Since part two of the Sakai et al. process fails to elute phosphatidylserine from the silica column even though products were known to be loaded onto the column, I performed some modifications to the elution step which were designed to elute phosphatidylserine product. For instance, I attempted eluting the column with 4:1 choloroform:methonal buffer, but still no phosphatidylserine eluted. I also tried eluting the column with water-

saturated chloroform-methanol buffer, but this buffer eluted phosphatidyl serine and phsophatidic acid in the same ratio in which they are present prior to the silica column. (See Figure 3).

In view of these experimental results, it is my opinion that a person of skill in the art cannot readily modify the Sakai et al. phosphatidylcholine purification process to obtain a phosphatidyl serine product having anything other than far inferior purity than the 95% purity level presently claimed in application 10/663,198.

It is further my opinion that the high degree of purity and yield of phosphatidylserine produced by the method described in patent application 10/663,198 results from the use of an enzymatic preparation of phospholipase D that has a transphophatidylation activity which is much higher than its hydrolyzing activity. For this reason, PA formation is very low and the PS/PA ration is very high (95:5) as seen in Examples 2, 3, 4 and 5 of application 10/663,198 .

The undersigned hereby declares that all statements made herein based upon knowledge are true, and that all statements made based upon information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

DATED: July, 6th 2007

Giampolo Menon

CURRICULUM VITAE

Name:	Giampaolo MENON
Place of birth:	Battaglia Terme (Padua) - Italy
Date of birth:	May 29th, 1961
Address:	Via Rocca Pendice, 6/B – Monteortone (Padua) Italy
Phone	+39/049/8669196
Email:	gmenon@fidiapharma.it
Education:	Diploma in Industrial Chemistry, 1980

Research and Professional Experiences:

1983 – 1991:	*Fidia Farmaceutici S.p.A. - Italy* Technician in the Laboratory of Chemical Research
1991 - 2001:	*Fidia Farmaceutici S.p.A. - Italy* Responsible of the R&D Laboratory – Main activities: Study and synthesis of new molecules, scale-up of processes regarding chemical and enzymatic synthesis, purification, analytical characterization and development of methods for the pharmacokinetic analysis of lipids, phospholipids, sphingolipids, estrogens, coumarines, azetidines, aminoacids, proteins, polysaccharides, vitamins, antioxidants, anti-inflammatory agents, gangliosides;
2001 - 2006:	*Fidia Farmaceutici S.p.A. - Italy* Analyst in the Quality Control Dept.
February 2006 - present	*Fidia Farmaceutici S.p.A. - Italy* Technician in the Laboratory of Analytical Development

Previous jobs:

1982 -1983:	Employee at *Sunchemical, Bologna - Italy*
1982	Sales man at *Würth, Italy*

Scientific Publications

- Chen Su, Menon G., Traldi P. (Organic Mass Spectrometry, 27, 215-218, 1992) "*Identification of aminophospholipid stereomers by positive ion fast atom bombardment combined with collisional activation mass-analysed ion kinetic energy analysis and HPLC*";

- Chen Su, Zangirolami L., Mariot R., Bellato P., Menon G., Traldi P. (The 39th ASMS conference on mass spectrometry and allied topics) "*Analysis of cloricromene acid levels in the rabbit plasma by continuous-flow fast atom bombardment (CF-FAB) mass spectrometry*";

- Chen Su, Curcuruto O., Catinella S., Menon G., Traldi P.(Biological Mass Spectrometry, 21, 655-666, 1992) "*Characterisation of the molecular species of glycerophospholipid from rabbit kidney: an alternative approach to the determination of the fatty acyl chain position by negative-ion fast atom bombardment combined with mass-analysed ion kinetic energy analysis*";

- Bevilacqua C., Menon G., Finesso M. and O'Regan M. (Journal of the Osteoarthritis Research Society International, Vol. 7, Suppl. A 120, 1999) "*Synovial fluid and plasma levels of bupivacaine after intraarticular administration in the presence of hyaluronic acid in rabbits*";

European Patents:

- Romeo A., Kirschner G., Menon G. - "*Therapeutical use of phosphoryl-L-serine-N-acyl-sphingosine*" – EP 0652755 B1;

- Kirschner G., Menon G., Vaccaro S. - "*Process for the preparation of pure phosphatides and their use in the cosmetic, pharmaceutical and alimentary fields*" – EP 1231213 B1;

- Prosdocimi M., Menon G., Monastra G., Galbiati E., Finesso M. - "*New coumarine derivatives and their salts, process for their production and their pharmaceutical use*" – EP 1307438 B1

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Updat. 27 June 2007

Figure 1:
results of TLC of Sakai's experiment 1:
starting from the bottom: 1° line: 40% of phosphatidyl choline
2° line: 40% of phosphatidyl serine
3° line: 15% phosphatidic acid with
two final spots

Column left Column right

Figure 2:

Column left: Fidia PS (95% PS and 5% PA)

Column right: eluate of purification step by silica column of Sakai's experiment 1

Figure 3:
Eluate of purification step by silica column using water saturated chloroform-methanol buffer:
Starting from the bottom: 1° line: phosphatidylserine
2°line: phosphatidic acid